ACUP Summary

Section BI - Base Information

Program(s)

(none entered)

1. Is this ACUP associated with Research Project?

No

2. ACUP Name

Please include the species and avoid abbreviations.

25-04-001 Validation of the Sperm Fertility Biomarker SP22 for Human Study using the Rabbit

3. Is there a previous ACUP/LAPR?

Yes

3.1. Previous ACUP/LAPR

22-04-001 Validation of sperm fertility biomarkers in the rabbit

4. Designation

Health

5. Does this protocol generate components for "non-animal models" such as cell or tissue culture?

No

6. Is this protocol a validation of a new approach method?

Yes

Principal Investigator

[redacted] (ORD-CPHEA-PHITD-RDTB)

Alternate Contact

[redacted] (ORD-CPHEA-PHITD-RDTB)

Status

Activated

Species

Rabbit

Strain

New Zealand White

Assigned to Agenda

03/23/2022

Section PD - Project Description

1. Research Project Description

Explain the study objective(s) in non-technical language such that it is understandable by non-scientific persons. Spell out all acronyms and abbreviations with their initial use.

For decades now the world-wide decline in human semen quality has been associated with exposure to environmental chemicals and pharmaceuticals. Unfortunately, we have not yet engaged in epidemiological study that can associate exposure with the adverse outcome of infertility. We are now tasked to apply our sperm biomarker of fertility (SP22) to human studies. The objective of this protocol is to use ejaculated rabbit sperm to assist in validating the use of our new recombinant monoclonal antibody to the SP22 protein. This antibody was custom synthesized and recognizes a 22 amino acid human sequence expressed over the equatorial segment. Thus far, we have found that the antibody recognizes the homologous sequence quite nicely on rabbit sperm (see image). However, significant challenges still lie ahead. Our current protocol was developed using freshly ejaculated rabbit sperm. We have not yet developed a protocol for frozen samples.

We recently launched 2 Material Transfer Agreements (MTAs) with human sperm biorepositories, one in Miami and one in Augusta. We have requested unidentified samples representative of both fertile and infertile donors. Naturally all these samples that we will be receiving will be frozen. Our next step is to cryopreserve ejaculated rabbit sperm similar to human sperm protocols. After removing the cryoprotectant we will initially attempt to repeat our fresh sperm staining protocol. Then we will try gradient isolation prior to staining as this is what most human sperm protocols recommend. Rabbit sperm allows us to develop the procedure for immunolocalization of the protein on frozen/thawed sperm before we begin to work with human samples.

2. Benefits of Proposed Research

Explain how the benefits from the knowledge gained from this research outweigh the costs to the animals used in this research. Does this benefit the species that will be used?

Senior management has requested this research. The MTAs have been approved and the work has been incorporated into a new StRAP4 project entitled "The Exposure Science of Reproductive Biofluids to Inform Hazard Identification and Risk Assessment". Our goal here is to process the samples using enzyme linked immunosorbent assay (ELISA) and

immunocytochemistry (ICC) for SP22. In addition to validating that SP22 expression is greater in fertile than infertile men we will proceed to submit sperm extracts via chemistry contract or in house for quantitative analysis of priority environmental chemicals and pharmaceuticals. By so doing we hope to be able to relate SP22 to reduced fertility of sperm contaminated with a given chemical paving the way for future epidemiology studies. Moreover, the EPA has an active patent for the use of SP22 in an in-home diagnostic kit to assess male fertility. The concept is to allow motile sperm to bind to an antibody-coated receptacle and relate the signal to a level of fertility. Our access to sperm from fertile and infertile men will facilitate the development and testing of such a kit. This work will not benefit or harm the rabbits used; however, the rabbits do like the attention they receive.

3. Research Project Approach

Describe the experimental design that will be used to meet the project objectives.

We currently have 3 male rabbits from which to collect sperm for in vitro experimentation. A "teaser" must be used to provide sufficient stimulation to mount and ejaculate. We have 1 old male to use as a teaser and we are planning on acquiring a custom plush New Zealand White dummy to use as a teaser. We now have 3 complete sets of artificial vaginas with separate collection tubes. The semen quality differs among the 3 young males. By collecting all 3 at a time we will be able to evaluate protocols without the day of collection being a confounder. We will probably need to collect the rabbits on a weekly basis until the protocols are developed. The StRAP4 proposal is in place for 3 years.

4. Assurance that Study is Not Unnecessary Duplication

Explain how it was determined that this is not unnecessary duplication. For novel work, a summary of literature search (including search engine, keywords, years covered) would be helpful. If this is a repetition of prior work, explain why this work is necessary.

A search of PubMed, Medline, etc. for human sperm and SP22 reveals no indication of duplication. Our recombinant antibody does not exist elsewhere. There is no data with this antibody other than what we generate.

5. Justify the continuation of the study

Justification is required if this protocol is related to a previously activated animal protocol.

Investigators in Brazil have used our antibody in an ELISA of fertile vs infertile human sperm. The data clearly indicates increased levels of SP22 in sperm from fertile men. But we still need to establish this relationship via immunolocalization on the sperm. As indicated above we are the only ones capable of achieving a protocol with this antibody that might be applicable to human sperm.

Scientific Rationale for Proposed Animal Use

6. Why is the use of animals necessary

Explain why this cannot be accomplished with a computer or other experiments.

There is no in vitro system to generate ejaculated sperm. The animals are only being used for sperm collection, and the sperm are used strictly for in vitro experimentation

7. Justify the species requested

The rabbit serves as a good model to study ejaculated sperm because the morphology of the sperm is quite similar to that of human sperm. Also, the rabbit is the preferred small animal laboratory model to study ejaculated sperm as it is impossible to noninvasively collect ejaculated sperm samples from rats or mice.

Section TM - Team Members

The team must comprise one active Principal Investigator and Alternate Contact to be complete. If team member does not appear in the menu, please add the new team member to the team.

Name	*Organization*	*Role*	*ORD Training*
[redacted]	ORD-CPHEA-PHITD-RDTB	Principal Investigator	Yes

Responsibilities

List specific techniques for which this team member has responsibility.

As Principal Investigator I am responsible for the research that Senior Management wants completed. I am solely responsible for the collection of ejaculates and subsequent testing.

Animal Training

Information related to Animal Training is associated with the specific team member. Please note that this information will affect other protocols for which the person is a team member.

Over 30 years of experience with rabbits

Name	*Organization*	*Role*	*ORD Training*
[redacted]	ORD-CPHEA-PHITD-RDTB	ACUP Alternate Contact	Yes

Responsibilities

List specific techniques for which this team member has responsibility.

Monitoring the rabbits weekly

Animal Training

Information related to Animal Training is associated with the specific team member. Please note that this information will affect other protocols for which the person is a team member.

30 years of experience with laboratory animals

Section PRD - In Vivo Procedures

1. Experimental Design Description

Provide the big picture for the study design here. The IACUC is looking to understand what will happen to the animals "cradle to grave". Describe cohorts, age/weight and sex of animals, the rationale and organization of proposed treatments and exposures, timelines and similar design items. Specific descriptions of procedures and justifications for animal numbers belong in different sections.

There are no in vivo exposures. The animals are used as sperm donors or teasers. The collected sperm are used only for in vitro experimentation. Rabbits will be collected on a weekly basis, typically 1 rabbit per week, but there will be times to collect all 3 during a visit to make sure the protocol works on samples of different quality.

2. Number of Animals Justification

We have 4 males, 3 young and 1 older.

Does this ACUP include any of the following

3. Restraint (> 15 min)

Describe how animals will be monitored, how health status will be tracked, and what records will be maintained.

No

4. Food and/or water restriction (>6 hrs)

Describe how animals will be monitored, how health status will be tracked, and what records will be maintained.

No

5. Survival surgery

Any surgery followed by deliberately allowing the animal to wake up. How long they survive after waking does not matter.

No

6. Non-survival surgery

Any surgical procedure where the animal is euthanized prior to waking. Terminal surgeries include procedures where the animal is alive only to allow organ function to make a procedure possible. Examples would be liver perfusion to collect hepatocytes or thoracotomy and subsequent blood collection from cardiac or aortic puncture.

No

Animal Pain/Distress Categories

7. Category B *Animals being bred, acclimatized, or held for use in teaching, testing, experiments, research, or surgery but not yet used for such purposes. Non-invasive observation only of animals in the wild.*

8. Category C *Animals that are subject to procedures that cause no pain or distress, or only momentary or slight pain or distress and do not require the use of pain-relieving drugs. Examples might be weighing an animal, or delivering an injection of a non-irritating substance.*

9. Category D *Animals subjected to potentially painful or stressful procedures for which they receive appropriate anesthetics, analgesics and/or tranquilizer drugs. An example would be survival surgery performed under anesthesia. Category D procedures require scientific justification and a search for alternatives.*

10. Category E *Animals subjected to potentially painful or stressful procedures that are not relieved with anesthetics, analgesics and/or tranquilizer drugs. Examples might include exposing animals to levels of pollutants which cause irritation of the eyes or respiratory tract; immobilization or paralysis of a conscious animal; application of noxious stimuli such as electrical shock that the animal cannot avoid/escape; continued research after clinical symptoms are evident without medical relief or requiring euthanasia. Category E procedures require scientific justification, including a justification for not using analgesics, anesthetics, etc., and a search for alternatives.*

	Adult	Offspring
7. Category B	0	0
8. Category C	4	0
9. Category D	0	0
10. Category E	0	0

Does this protocol include any of the following procedures?

12. Category C Procedures

Yes

13. Non-Surgical Category D&E Procedures

No

14. Surgical Category D&E Procedures

No

12. Category C Procedures

12.1. Treatments

List dosages, duration of exposure, route, volume, and frequency.

A least one rabbit will most likely be collected each week. We now have the ability to collect all 3 young males during one visit.
Ejaculates are collected using a pre-fabricated, water-jacketed artificial vagina and a teaser. The water in the water jacket does not exceed 40 degrees C. The teaser is brought into the male's cage and the artificial vagina is placed under the belly of the male, positioned between the male and the teaser when mounting begins.

12.2. Survival Blood Collection

List method, volume, and frequency.

N/A

12.3. Terminal Blood Collection

N/A

12.4. Testing Methods

Include non-stressful dietary restrictions/modifications, mild non-damaging electric shock.

N/A

12.5. Animal restraint and confinement beyond routine housing and handling.
Include a description of the type of restraint device, acclimation to device, duration of restraint.

N/A

12.6. Breeding for experimental purposes
Information can include length of pairing, number of generations, etc.

N/A

12.7. Describe how animals will be identified and monitored
Include description of identification procedures. (For example, if transponders are used, how are the animals prepared?) Include frequency of observations and by whom.

Rabbits are identified by name as well as number on the cage card; animal numbers are maintained on the ear with indelible marker. The rabbits are monitored once a week by laboratory staff.

15. Humane Interventions

15.1. Resultant effects
Do the investigators expect to see following procedures or treatment? Please include transitory as well as permanent effects. Examples might include lethargy, ataxia, salivation or tremors. Indicate the expected duration of these effects.

We don't expect to see any physical issues with the younger males. Eventually the older male will lose more weight even if he continues to eat well. This will represent muscle loss. At this point he will be given critical care slurry. Their feet are well-maintained by the animal care staff.

15.2. State the criteria for determining temporary or permanent removal of animals from the study
Describe actions to be taken in the event of deleterious effects from procedures or chemical exposures. Describe actions to be taken in the event of clinical health problems not caused by procedures or exposures.

Animal care notifies us and the Veterinarian if an animal starts to develop a problem such as loss in appetite and/or weight. Common health issues that may occur in rabbits include: adenocarcinoma, bladder sludge, gastric stasis and pododermatitis. Pododermatitis is treated with increased exercise in the exercise pen, and both monitoring and treatment by the Attending Veterinarian as needed. Bladder sludge and gastric stasis can be treated with fluids, analgesics, and a special diet (a critical care herbivore slurry we have on site). Adenocarcinoma requires the animal be spayed or euthanized. Regardless of the disorder, the Attending Veterinarian is consulted. If quality of life is compromised the animal is euthanized.

Section REQ - Animal Requirements

1. Animals to be purchased from a Vendor for this study

0

2. Animals to be transferred from another ACUP

4

2.1. ACUP that is the source of the transfer

22-04-001 - Validation of sperm fertility biomarkers in the rabbit

3. Offspring produced onside (used for data collection and/or weaned)

0

Total Number of animals for duration of the ACUP

4

4. Species (limited to one per ACUP)

Rabbit

5. Strain(s)

New Zealand White

6. Describe special requirements for animals with altered physiological responses

N/A

7. Sources of Animals

Other

7.1. Describe your Animal Source

Covance

8. Will any animals be housed in areas other than the animal facility longer than 12 hrs

No

9. Housing or Husbandry Requirements/Housing and Enrichment

Rabbits are single housed to avoid fighting between intact males. Adjacent cages are open for access from one side to the other. Environmental enrichment includes regular exercise in the exercise area, bathing, and toys. New food items such as hay, vegetables, and fresh greens are provided as supplemental diet enrichment. Critical care slurry is provided to all rabbits when needed.

10. Special Assistance Requested

Please describe any Technical Service Requests which may be submitted to the Animal Resources Program Office to obtain Animal

Care Staff assistance with animal procedures. Technical Service Requests can be submitted for such diverse assistance as assisting with oral lavage dosing or vaginal lavage, assistance transporting animals to or from High Bay, assistance with maintaining the fish breeding colonies, or assistance performing euthanasia. NOTE: This request must be submitted separately to the Animal Resources Program Office (ARPO)

N/A

11. Laboratories where Animal Procedures will be Conducted

N/A

Section AS - Additional Species

No Additional Species

Section AA - Agents Administered to Animals

As defined by the ORD SHEMF Office, a particularly hazardous agent exhibits one or more of these characteristics

- OSHA (GHS) Acute Toxicity Hazard Categories 1 and 2
  - Has an oral LD50 acute toxicity value (rat) $\leq$ 50 mg/kg body weight
  - Has an inhalation LC50 acute toxicity value (rat) $\leq$ 2 mg/liter or $\leq$ 500 ppm
  - Has a dermal LD50 acute toxicity value (rat or rabbit) $\leq$ 200 mg/kg body weight
  - Has an occupational exposure limit (OSHA, NIOSH or ACGIH) $\leq$ 1 ppm
- Causes carcinogenic effects (confirmed or suspected in humans and/or confirm animals) **- OSHA (GHS) Carcinogen Categories 1 and 2**
- Causes teratogenic, mutagenic or reproductive effects (in humans or animals) **- OSHA (GHS) Categories 1 and 2 for Germ Cell Mutagenicity and Reproductive Toxicity**
- Is an infectious biological agent, including human cell lines, human blood, or other potentially infectious materials (as defined by CDC and/or NIH)
- Is an explosive or violently reactive agent (shock sensitive, peroxide-forming (Category A-forming explosive levels of peroxides without concentration), and/or incompatible with moisture/air
- Is a respiratory or skin sensitizing agent
- Nanoparticle research involving the use of manufactured nanoparticles (metal oxides, carbon nanotubes, nano silica, etc.) not contained in solution and/or with the possibility of airborne exposure
- Is an agent whose toxicological characteristics are unknown, but it is suspected of meeting one of the above criteria

EXCEPTION: Standards ordered from vendors in sealed vials or ampoules that used directly in laboratory instrumentation are exemption even if they meet the above criteria.

1. Describe safety precautions for agents not covered by HSRP

N/A

2. Do you plan to administer human or animal tissues, or body fluids?

No

Section ABC - Animal Breeding Colonies

1. Does this ACUP have animal breeding colonies:

No

Section EU - Euthanasia

1. When will the animals be euthanized relative to experimental procedures?

Please make sure all categories of animals are covered.

An animal will be euthanized as per Attending Veterinarian recommendations if a health issue than can't be managed

Chemical Euthanasia Methods
Physical Euthanasia Methods

2. Describe the disposition of any animals remaining after project completion

Euthanized by the veterinarian.

3. Would you consider transferring any unused animals from this ACUP to another approved ACUP

The IACUC encourages investigators to reduce the overall number of animals used

Yes

Section ASR - Assurances

Name	*Organization*	*Role*	*Date*
[redacted]	ORD-CPHEA-PHITD-RDTB	Principal Investigator	03/03/2022

- Animals will not be used in any manner beyond that described in this application without first obtaining formal approval of the IACUC.
- All individuals involved in this project have access to this application, are aware of all EPA policies on animal care and use, and are appropriately trained and qualified to perform the techniques described.
- Thorough consideration of the three "R"'s (Replacement, Reduction, Refinement) has been given, as applicable, to a. the use of animals, and b. procedures causing pain or distress (with or without analgesia/anesthesia), including death as an endpoint. The minimum number of animals required to obtain valid experimental results will be used.
- The Attending Veterinarian has been consulted in regard to any planned experimentation involving pain or distress to animals.
- The IACUC and Attending Veterinarian will be promptly notified of any unexpected study results that impact the animals' well-being, including morbidity, mortality and any occurrences of clinical symptoms which may cause pain or indicate distress.
- All procedures involving hazardous agents will be conducted in accordance with practices approved by the Safety, Health, and Environmental Management Office.
- I certify that I am familiar with and will comply with all pertinent institutional, state and federal rules and policies.
- The IACUC has oversight responsibilities for animal care and use, and may request consultation or feedback regarding the conduct of in vivo procedures, progress and accomplishments, and any problems encountered.

Section REV - Reviewers

Name	*Role*
[redacted] (ORD-CPHEA-PHITD-RDTB)	Branch Chief
Leslie Jarrell (ORD-ORM-RSCD-FRCB)	Attending Veterinarian

Section SIG - IACUC Signatures

Name	Role	Date
Leslie Jarrell (ORD-ORM-RSCD-FRCB)	Attending Veterinarian	03/31/2022 04:28 PM

Comments
DMR

Name	Role	Date
[redacted] (ORD-CPHEA-PHITD-RDTB)	IACUC Member	03/31/2022 01:41 PM

Comments
Approved by DMR

Section ATT - Attachments

File Name	File Type	Upload Date	Uploaded By
Bunnies-Recombinant	PowerPoint Document	03/03/2022	[redacted] (ORD-CPHEA-PHITD-RDTB)
Journal of Andrology - 2013 - Amann - Artificial Vagina for Rabbits	PDF	03/03/2022	[redacted] (ORD-CPHEA-PHITD-RDTB)

Section HIS - Status History

Name	*Transition*	*Status Changed To*	*Date/Time*
[redacted] (ORD-ORM-RSCD-FRCB)	Activate ACUP	Activated	03/31/2022 08:25 PM

Comments

Activating as DMRs have signed.

Name	*Transition*	*Status Changed To*	*Date/Time*
[redacted] (ORD-ORM-RSCD-FRCB)	Begin DMR	In Designated Member Review	03/31/2022 08:24 PM

Comments

Name	*Transition*	*Status Changed To*	*Date/Time*
[redacted] (ORD-CPHEA-PHITD-RDTB)	Submit ACUP	Pending Designated Member Review	03/25/2022 09:07 AM

Comments

Name	*Transition*	*Status Changed To*	*Date/Time*
[redacted] (ORD-CPHEA-PHITD-RDTB)	Unlock ACUP	In Progress: Pending Designated Member Review	03/25/2022 09:03 AM

Comments

Name	*Transition*	*Status Changed To*	*Date/Time*
[redacted] (ORD-CPHEA-PHITD-RDTB)	Submit ACUP	Pending Designated Member Review	03/24/2022 03:06 PM

Comments

Name	*Transition*	*Status Changed To*	*Date/Time*
[redacted] (ORD-ORM-RSCD-FRCB)	Request Revisions for DMR	In Progress: Pending Designated Member Review	03/23/2022 05:05 PM

Comments
DMR

Name	*Transition*	*Status Changed To*	*Date/Time*
[redacted] (ORD-ORM-RSCD-FRCB)	Assign to Agenda	In Full Committee Review	03/14/2022 06:53 AM

Comments

Name	*Transition*	*Status Changed To*	*Date/Time*
[redacted] (ORD-CPHEA-PHITD-RDTB)	Approve Draft ACUP	Pending Agenda Assignment	03/04/2022 11:59 AM

Comments
As AO, I approve this request.

Name	*Transition*	*Status Changed To*	*Date/Time*
[redacted] (ORD-CPHEA-PHITD-RDTB)	Begin BC Review	In Branch Chief Review	03/04/2022 11:59 AM

Comments
As AO, I approve this request.

Name	*Transition*	*Status Changed To*	*Date/Time*
[redacted] (ORD-CPHEA-PHITD-RDTB)	Submit ACUP	Pending Branch Chief Review	03/03/2022 11:57 AM

Comments

Name	***Transition***	***Status Changed To***	***Date/Time***
[redacted] (ORD-CPHEA-PHITD-RDTB)	Create ACUP	In Progress	03/02/2022 11:40 AM

Comments